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**THORACIC CHAETOTAXY IN ELEVEN MOSQUITO SPECIES
(DIPTERA: CULICIDAE) OF THE GENERA *ANOPHELES* MEIGEN,
1818, *URANOTAENIA* LYNCH ARRIBALZAGA, 1891, *CULISETA* FELT,
1904, *COQUILLETIDIA* DYAR, 1905, *CULEX* LINNAEUS, 1758,
AND *LUTZIA* THEOBALD, 1903 : DESCRIPTION OF VARIABILITY
WITH CONSIDERATION OF ASYMMETRY**

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The setation variability was studied for the postpronotal, prespiracular, prealar, upper and lower mesepisternal, and upper and lower mesepimeral setae in several species of the genera *Anopheles* Meigen, 1818, *Uranotaenia* Lynch Arribalzaga, 1891; *Culiseta* Felt, 1904; *Coquillettidia* Dyar, 1905, *Culex* Linnaeus, 1758, and *Lutzia* Theobald, 1903. The new traits which allow to distinguish the species by setation were found. Among them are the presence of prespiracular setae in *Anopheles claviger*, a single postpronotal, prealar, prespiracular, and the lower mesepimeral seta in *Uranotaenia unguiculata*. Moreover, significant differences in the number of setae were revealed in many comparisons between several species, but, in most cases, with the notable overlap. We also found considerable differences between sexes in the number of the upper mesepimeral setae in *Coquillettidia richiardii*, *Culex modestus*, *Culex hortensis*, and *Culiseta alaskaensis indica*, and the postpronotal setae in *Culex theileri*. Predominantly left-lateralized distribution of the postpronotal setae has been identified for *Coquillettidia richiardii* and predominantly left-lateralized distribution of the lower mesepisternal setae has been detected for *Culex modestus* (taking into account the magnitude of bias, the leftward asymmetry on the species level was confirmed statistically with $p < 0.05$).

Key words: thoracic chaetotaxy, laterality, sexual dimorphism, Culicidae, mosquito.

ХЕТОТАКСИЯ ГРУДИ 11 ВИДОВ ИЗ РОДОВ *ANOPHELES* MEIGEN, 1818,
URANOAEIA LYNCH ARRIBALZAGA, 1891, *CULISETA* FELT, 1904,
COQUILLETTIDIA DYAR, 1905, *CULEX* LINNAEUS, 1758
И *LUTZIA* THEOBALD, 1903 КРОВОСОСУЩИХ КОМАРОВ
(DIPTERA: CULICIDAE): ОПИСАНИЕ ВАРИАБЕЛЬНОСТИ
С РАССМОТРЕНИЕМ АСИММЕТРИИ

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Изучена изменчивость постпронотальных, дыхальцевых, преаларных, верхних и нижних мезэпистернальных, верхних и нижних мезэпимерных щетинок у нескольких видов родов *Culex* Linnaeus, 1758; *Lutzia* Theobald, 1903; *Uranotaenia* Lynch Arribalzaga, 1891; *Culiseta* Felt, 1904; *Coquillettidia* Dyar, 1905 и *Anopheles* Meigen, 1818. Были найдены новые признаки, позволяющие различать виды по расположению и количеству щетинок. Среди них наличие дыхальцевых щетинок у *Anopheles claviger*, по одной постпронотальной, преаларной, дыхальцевой и нижней мезэпимерной щетинке у *Uranotaenia unguiculata*, а также значимые различия в количестве щетинок, выявленные во многих сравнениях между несколькими видами, но с заметным перекрыванием в большинстве случаев. Мы также обнаружили значимые различия между полами в числе верхних мезэпимерных щетинок у *Coquillettidia richiardii*, *Culex modestus*, *Culex hortensis* и *Culiseta alaskaensis indica* и постпронотальных щетинок у *Culex theileri*. Вид *Coquillettidia richiardii* имеет преимущественно левостороннюю асимметрию по числу постпронотальных щетинок, *Culex modestus* латерализован преимущественно влево по числу нижних мезэпистернальных щетинок (с учетом величины латеральности, преобладание левосторонней асимметричности на видовом уровне было подтверждено статистически с $p < 0.05$).

Ключевые слова: хетотаксия груди, латеральность, половой диморфизм, Culicidae, кровососущие комары.

Mosquitoes (Diptera Linnaeus, 1758; Culicidae Meigen, 1818) are a biodiversity-relevant taxonomic category that represents a significant fraction of hematophagous insects in the world. Females of many mosquito species, being active human biters, can cause a serious nuisance close to the human recreation areas and the work places. A number of human and/or nonhuman parasites use female mosquitoes as vectors to spread. The Culicidae are responsible for the transmission of vector-borne pathogens that cause diseases, including malaria, filariasis (dog heartworm), and viral diseases such as dengue, Japanese encephalitis, and yellow fever (Becker et al., 2010). The mosquito-borne hemorrhagic fevers sometimes become a problem in northern regions: for instance in Russia the Karelian fever was recorded in the Republic of Karelia, Vologda and Smolensk Provinces (L'vov et al., 1989). The vector competence of different mosquito species for vector-borne pathogens is not always evident (Leggewie et al., 2016). Thus, an accurate identification of the mosquito specimens to the species level can contribute to better insights into the vector-pathogen interactions. In

view of this, we applied precise diagnostic technique for detecting and assessing the additional inter- and intraspecific traits of the Culicidae species.

The genital morphology studies are appropriate for species identification of adult male mosquitoes, whereas the species identification of adult females is substantially based on other morphological traits including thoracic chaetotaxy. The thoracic sclerites are the least damaged structures after collection and storage of the specimens (particularly in pinned mosquitoes the lateral thoracic sclerites are often intact). The scales and setae are often lost after dry storage or fixation in ethanol, but their bases are well-defined on the sclerite surface under the light microscopy (LM). Therefore, the thoracic chaetotaxy elements may be useful to discriminate mosquito species independently of sex.

According to a revised sclerite terminology (Khalin, Aibulatov, 2014), which allows to avoid omonymy, thoracic subdivisions of culicids include the pronotum (subdivided into anteropronotum and postpronotum), propleuron, postprocoxal membrane, prosternum, anteroprocoxal membrane, mesonotum (includes prescutum, scutum, scutellum, and postnotum), mesopleuron (subdivided into mesepisternum and mesepimeron; in turn, mesepisternum is divided into mesanepisternum and mesokatepisternum; analogously, mesepimeron is divided into mesanepimeron and mesokatepimeron), mesotrochantin, mesomeron, mesosternum, metanotum, metapleuron (divided into metepisternum and metepimeron), metameron, and metasternum (fig. 1, see ins.). Setae and scales form specific areas on thoracic subdivisions. In our opinion, the most analytically favorable of them are several lateral groups of setae, i. e. postpronotal (upper and posterior), prespiracular, prealar, mesepisternal (upper and lower), and mesepimeral (upper and lower) setae (Khalin and Aibulatov, 2016) (fig. 2).

There are many examples of deviations from the bilateral symmetry in morphological traits of insects. Well-known examples concerning external morphology include the asymmetric shape of genitalia in Dictyoptera, Phasmatodea, Embiidina etc. (Huber et al., 2007) or structural nonequivalence of forewings in males of many orthopterans. Examples of an asymmetric internal morphology, such as biased location of the retrocerebral glands in crickets (Razygraev, 2013), are also known. The high frequency of asymmetric setation in mosquitoes has been noticed in this study, based on previous surveys (Khalin, Aibulatov, 2016). We suggested that the asymmetry of setation might be especially important during species identification of the specimens with damaged left or right side of the thorax.

In a previous study, we elaborated a preparation technique allowing to analyze the thoracic pleurite chaetotaxy with LM using pinned and ethanol-stored specimens as an initial material (Khalin, Aibulatov, 2012). In the present study, using this technique, we investigated the number and distribution of setae on lateral thoracic sclerites in different Culicidae genera and species to elaborate additional criteria for genera and species diagnostics. Thus, the following tasks were set: (1) to evaluate the variability of the number and distribution of thoracic setae in several species of the genera *Culex* Linnaeus, 1758, *Lutzia* Theobald, 1903, *Uranotaenia* Lynch Arribalzaga, 1891, *Culiseta* Felt, 1904, *Coquillettidia* Dyar, 1905, and *Anopheles* Meigen, 1818; (2) to perform statistical comparisons between each species taking into consideration the number of setae; and (3) to assess a possible species-level (intraspecific) asymmetry of setae

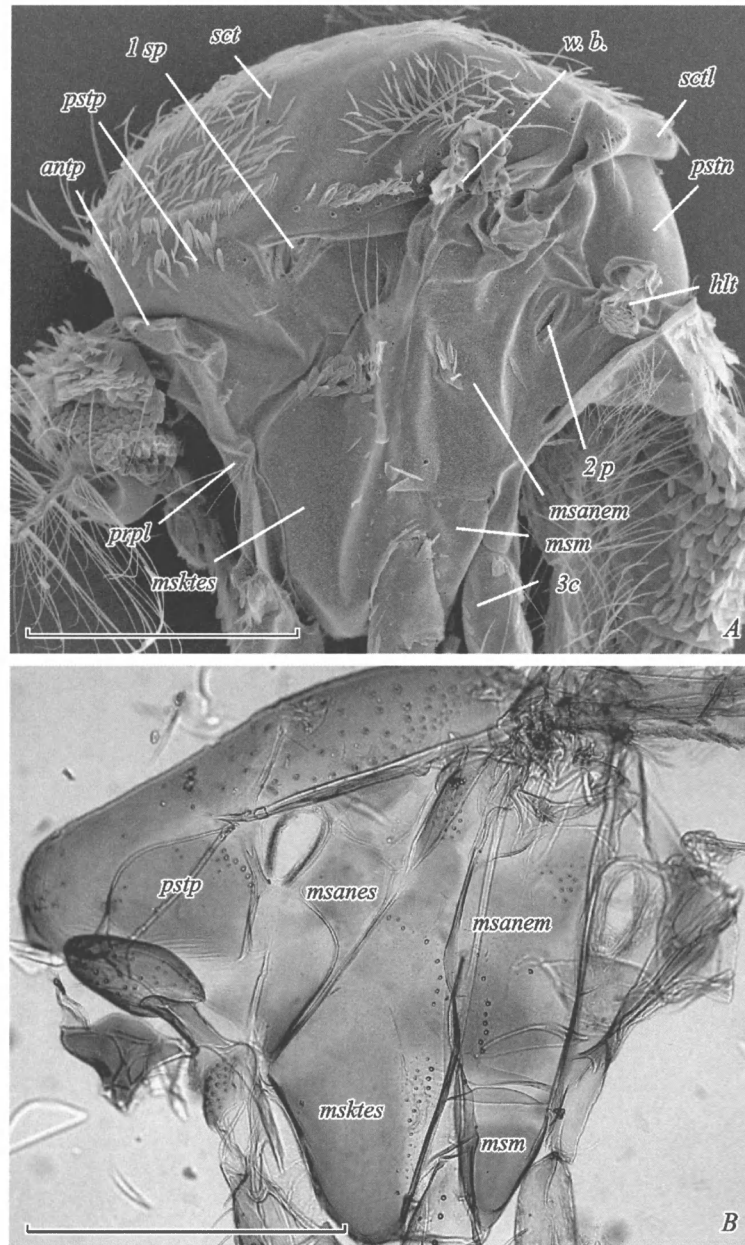


Fig. 1. Culicidae Meigen, 1818, female thorax in lateral view.

A — *Uranotaenia unguiculata* Edwards, 1913, SEM; *B* — *Lutzia halifaxii* (Theobald, 1903), LM. Prothorax: *antp* and *pstp* — antero- and postpronotum, *prpl* — propleuron. Mesothorax: mesonotum: *set* — scutum, *scutl* — scutellum, *pstn* — postnotum; mesopleuron: *msanes* and *msktes* — mesanepisternum and mesokatepisternum, *msanem* — mesanepimeron, *msm* — mesomeron. *1s* and *2s* — 1st and 2nd thoracic spiracles, *1c* and *3c* — procoxa and metacoxa, *w.b* — wing base, *hlt* — halter [terminology after Khalin and Aibulatov (2014)]. Scale bar, mm: *A* — 0.5, *B* — 1.

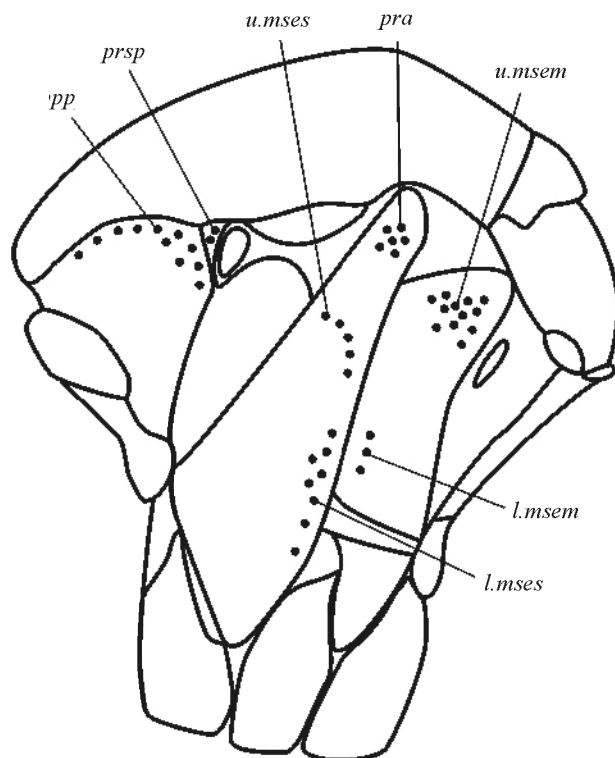


Fig. 2. Culicidae Meigen, 1818, setation scheme of the adult mosquito thorax, lateral view.
pp — postpronotal, *pra* — prealar, *prsp* — prespiracular, *u.msem* and *l.msem* — upper and lower mesepimeral,
u.mses and *l.mses* — upper and lower mesepisternal [terminology after Khalin and Aibulatov (2014)].

distribution by paired comparisons of the left and right pleurites with each other on the basis of setal number.

The geographical distribution of the samples prepared and analyzed in the present study is limited by Russian Federation and several adjacent and nearby territories.

MATERIALS AND METHODS

The study was performed in the Laboratory of Parasitology of the Zoological Institute, Russian Academy of Sciences, St. Petersburg. We used material from the collection of the Zoological Institute (pinned adults) as well as our own collected material. A total of 258 specimens of 11 species (6 genera) were included in the study of thoracic chaetotaxy. In total, 127 specimens belonged to the Zoological Institute collection (10 species) and 131 specimens were collected by the authors (4 species). Overall, 228 specimens of 11 species were studied using LM and 30 specimens of 8 species were studied using scanning electron microscopy (SEM).

The species investigated and the regions of the origin of specimens are listed below:

Anopheles (*Anopheles*) *claviger* (Meigen, 1804): in Russia, specimens were collected in Leningrad and Rostov Provinces, Krasnodar and Stavropol Territo-

ries; in Ukraine, in Lviv and Dnepropetrovsk regions. Material was also collected in Georgia, Turkmenistan, and Kyrgyzstan. 9♂, 14♀. *Anopheles (Cellia) pulcherrimus* Theobald, 1902: specimens were collected in Kazakhstan, Turkmenistan, and Uzbekistan. 8♂, 13♀. *Coquillettidia (Coquillettidia) richiardii* (Ficalbi, 1889): own collections — 39 specimens, 11♂, 28♀ (in Russia — Leningrad and Samara Provinces; in Ukraine — Kherson region). 3 specimens (3♂, Leningrad Province) from the Zoological Institute collection were also examined. *Culex (Barraudius) modestus* Ficalbi, 1890: 22 specimens (11♂, 11♀) from Russia (Ulyanovsk Province, Stavropol Territory, and Primorye), Ukraine (Kherson region), Kazakhstan, Kyrgyzstan, and Uzbekistan. *Culex (Culex) pipiens* Linnaeus, 1758: own collections, 32 specimens (13♂, 19♀), Russia (Leningrad Province). *Culex (Culex) theileri* Theobald, 1903: 11 specimens (5♂, 6♀) from the Zoological Institute collection from Armenia, Tajikistan, and Russia (Stavropol Territory). *Culex (Maillotia) hortensis* Ficalbi, 1889: 12 specimens (4♂, 8♀) from the Zoological Institute collection from Russia (Crimea) and Turkmenistan. *Culex (Neoculex) territans* Walker, 1856: The total number of specimens is 23 (10♂, 13♀). Own collections from Russia (Leningrad Province) and materials from the Zoological Institute collection from Russia (Leningrad Province and Stavropol Territory, Primorye, and the Mari El Republic) and Ukraine (Kiev region). *Culiseta (Culiseta) alaskaensis alaskaensis* (Ludlow, 1906): the total number of specimens is 21 (7♂, 14♀). Our own collections are from Russia (Leningrad Province, 5♀); other materials are from the Zoological Institute collection from Russia (Leningrad Province, Stavropol and Khabarovsk Territories, and Kamchatka) and from Uzbekistan. *Culiseta (Culiseta) alaskaensis indica* (Edwards, 1920): material from the Zoological Institute collection from Tajikistan and Iran, 10 specimens (4♂, 6♀). *Lutzia (Metalutzia) halifaxii* (Theobald, 1903): 7 specimens (3♂, 4♀) from the Zoological Institute collection from Russia (Primorye). *Uranotaenia (Pseudoficalbia) unguiculata* Edwards, 1913: 10 specimens (4♂, 6♀) from the Zoological Institute collection from Kazakhstan and Uzbekistan.

The methods of LM with the computer processing of the images obtained were used. Leica MZ95 and Leica DM5000B optical microscopes were used; images captured with MC-6.3 and Leica DFC320 digital cameras were processed using Adobe Photoshop CS5.1, Corel Draw X6, and Helicon Focus 6 software.

The treatment and mounting of the specimens were previously described in detail (Khalin, Aibulatov, 2016). Briefly, prior to LM examination, an adult mosquito was treated with 10 % KOH solution in water, then the thorax was dissected sagittally and both halves were rinsed to remove the remains of the internal structures and embedded on slides as permanent or temporary mounts. In large specimens, the most part of the mesonotum was removed to optimize the placement of pleurites in the slide. To prepare the permanent slides, the specimens were treated by a graded ethanol series, lavender oil, and xylene, and then embedded in Canada balsam. Temporary mounts were embedded in 70 % glycerol solution in water.

The objects were prepared for SEM as follows. The specimens were dissected with tungsten needles, microsurgical scissors, and tweezers under a Leica MZ95 binocular microscope, then mounted on stubs with a glue or two-sided adhesive tape and coated with platinum in a vacuum chamber. The chaetotaxy elements (setae and scales) were removed by a specially prepared mini brush

with 5—7 short hairs to make the bases of setae well-defined on the sclerite surface. The images were obtained with SEMs (Hitachi TM-1000, Hitachi S 570, and FEI Quata-250) using an ETD detector.

Species comparisons

Setal number of each group of setae was tested for normality of underlying distribution using a Shapiro—Wilk test (the most powerful normality test according to Razali et Wah (2011)) prior to proceeding the description and comparisons. Since deviations from normality were revealed, the sample description was performed using medians (Me) and the lower and upper values (min—max) for a sample (*min—max (Me)*, see table), and comparisons were carried out with a Wilcoxon—Mann—Whitney test with continuity correction. All «observations» in samples that were compared were averages between left and right sides of specimens. Me and min—max presented in the main text of the article were calculated on the basis of these averages (for the full set of these data, see Khalin, Aibulatov, 2016).

In cases of asymmetric distributions of setae, the range of the number of setae can be wider, if calculated using data for each particular side and independently of the side. Thus, the Me and min—max values for each side (left and right) were also calculated (see table). The min—max range independently of the side can be easily estimated using the table.

Setation asymmetry assessment

Each certain group of setae on the left sclerite was compared with its right counterpart using the setal number. The pairwise comparisons were performed using a Wilcoxon signed rank test (with continuity correction) to test the null hypothesis, according to which the setae on the certain location are equally likely to be prevalent on the left and on the right side of the thorax (taking into account the magnitude of differences between the left and right sides). The fraction of symmetric specimens in a sample was indicated if the significant leftward or rightward lateralization was revealed. The laterality indices ($((\text{left} - \text{right})/(\text{left} + \text{right}))$) are also presented in such cases. Asymmetry estimation was performed independently of sex.

Significance estimation

Calculations of the type I error probabilities were performed in R environment for statistical computing (version 2.13.1, R Core Team, 2011). The Holm-Bonferroni correction procedure (Holm, 1979) was applied to the p-value taking into account the fact that the various samples were compared repeatedly (p' means corrected p-value). In the study of intra- and interspecific variability, various mosquito species were compared using in this case the most analytically favorable, not all possible parameters of setation, and the Holm-Bonferroni correction procedure was applied correspondingly. When asymmetry was estimated, the Holm-Bonferroni correction procedure was applied to the results of all pairwise (left-right) comparisons performed on the most analytically preferable setae groups chosen beforehand, in which the setae number is not equal to zero.

Number of setae of the examined mosquito species

[illegible]

<i>Culex hortensis</i>	4—8 (6)	4—9 (6)	0	0	6—15 (9)	6—14 (9)	1—2 (2)	1—3 (2)	4—11 (8)	4—11 (7)	8—28 (18)	7—28 (18.5)	1—2 (1)	1—2 (1)
♂♂											8—13 (10.5)	7—11 (10)		
♀♀											13—28 (22)	17—28 (24)		
<i>C. territans</i>	5—12 (8.5)	5—12 (8)	0	0	6—14 (9)	5—14 (11)	4—7 (5)	3—7 (5)	4—10 (7.5)	3—10 (6.5)	7—19 (13)	7—20 (14)	1	1—2 (1)
<i>Culiseta alaskaensis alaskaensis*</i>	8—15 (11.5)	8—14 (10)	12—20 (15)	11—19 (16)	18—28 (22)	16—29 (23)	5—10 (7)	5—10 (7)	10—22 (16)	8—21 (17)	15—28 (24)	14—31 (22.5)	1—8 (5)	1—7 (5)
♂♂													1—5 (2.5)	1—5 (2)
♀♀													4—8 (5.5)	4—7 (5)
<i>C. alaskaensis indica</i>	3—8 (7)	4—9 (6)	7—14 (10)	4—16 (10)	8—23 (15)	9—23 (17)	2—8 (6)	3—6 (5)	8—14 (12)	5—17 (10.5)	10—21 (17.5)	8—22 (15.5)	2—5 (3)	0—4 (3)
♂♂					8—14 (12)	9—14 (12)					10—16 (13)	8—15 (10.5)		
♀♀					15—23 (20)	17—23 (19)					17—21 (18)	15—22 (19)		
<i>Lutzia halifaxii</i>	9—18 (13)	8—16 (15)	0	0	20—32 (26)	20—32 (25)	5—9 (7)	6—9 (7)	11—17 (15)	12—20 (16)	15—25 (18)	16—24 (20)	6—10 (9)	7—10 (7)
<i>Uranotaenia unguiculata</i>	1	1	1—2 (1)	1	1	1	4—7 (6.5)	5—7 (5)	3—9 (7.5)	5—10 (6)	5—10 (7.5)	5—8 (6)	1	1

Note. The given values correspond to the minimum-maximum range and, in parentheses, the median (min—max (Me)). * — in this sample, the only one specimen (1♂, Uzbekistan) had only right thoracic sclerites available for analysis with the lowest number of postpronotal (7), prespiracular (4), prealar (13), and upper and lower mesepimeral setae (12 and 0, respectively), and was removed from the analysis. The data on males and females were presented only in a combined form, if the sexual dimorphism was not revealed.

RESULTS

Intra- and interspecific variability of setation

Most of the results on min—max ranges (and medians) calculated using averages for the left and right sides of each specimen, as well as the results of statistical comparisons (p' -values), are presented below. The complete results regarding the average values for the left and right sides and their statistical comparisons are available (Khalin, Aibulatov, 2016). The variabilities of setation analyzed separately for the left and right sides are presented here in table.

Postpronotal setae are absent in both species of the genus *Anopheles*. In *Coquillettidia richiardii*, there are 5—14 (Me = 9) postpronotal setae arranged in 1—2 lines along the upper and posterior edges of the postpronotum; the most part of them is arranged in a slanting line. *Culex modestus*, *C. hortensis*, *C. pipiens*, *C. territans*, and *C. theileri* (ordered by ascending median number of setae) have 2.5—6.5 (Me = 3.5), 4.5—8.5 (Me = 6), 4—12 (Me = 7.5), 5—12 (Me = 8), and 7—10.5 (Me = 8.5) postpronotal setae, respectively. The significant differences were found after the comparison of *C. theileri* with *C. hortensis* and *C. modestus* by this parameter, with $p' < 0.001$ and $1 \cdot 10^{-4}$, respectively. *Lutzia halifaxii* has 8.5—17 (Me = 14) postpronotal setae and significantly differs from each of the first four species of the genus *Culex*, listed above, by this parameter with $p' < 0.001$. Two subspecies of *Culiseta alaskaensis* significantly differ from each other in the number of postpronotal setae with $p' < 1 \cdot 10^{-4}$; values were 4—8.5 (Me = 6.75) and 8—13.5 (Me = 10.5) for *C. a. indica* and *C. a. alaskaensis*, respectively. The postpronotal setae are arranged in 1—2 lines along the upper and posterior edges of the postpronotum in all five species of the genus *Culex*, *Lutzia halifaxii*, and *Culiseta alaskaensis*. All the specimens of *Uranotaenia unguiculata* studied have a single seta on the postpronotum.

Prespiracular setae are absent in all studied species of the genus *Culex*. They are also absent in *Lutzia halifaxii*, *Coquillettidia richiardii*, and *Anopheles pulcherrimus*. *Anopheles claviger* has 4—10 (Me = 7.5) prespiracular setae arranged in 2—3 irregular lines along the anterior edge of the mesothoracic spiracle. *Uranotaenia unguiculata*, in general, has a single prespiracular seta located anterior to spiracle; one specimen had a second, thinner, prespiracular seta on the right side. In *Culiseta alaskaensis*, the prespiracular setae are arranged in 1—2 (*C. a. indica*) or 1—3 (*C. a. alaskaensis*) lines along the anterior edge of the spiracle; their average number is between 4.0 and 19.5; *Culiseta a. indica*, in general, has a lower number of prespiracular setae than *C. a. alaskaensis*.

Prealar setae in all species studied (except *Uranotaenia unguiculata*) are arranged in an irregular group. In *Anopheles claviger* and *A. pulcherrimus*, the number of prealar setae was 5.5—11.0 (Me = 8.0) and 4.5—9.5 (Me = 6.25), respectively, that is significantly lower (with $p' < 1 \cdot 10^{-7}$ in both comparisons) than the same parameter in *C. alaskaensis* (subspecies were not separated for statistical comparisons). This parameter is sexually dimorphic for *C. a. indica* (see below under «Sexually dimorphic setation» subheading); for *C. a. alaskaensis*, the values were 17.0—28.3 (Me = 23). Subspecies of *C. alaskaensis* also differ by this parameter ($p' < 0.001$): the median number is greater in *C. a. alaskaensis*. *Culiseta alaskaensis* has significantly higher number of prealar setae in comparison with *Culex pipiens* ($p' < 1 \cdot 10^{-8}$), *Culex modestus* ($p' < 1 \cdot 10^{-7}$),

and *Culex territans* ($p' < 1 \cdot 10^{-6}$); the latter three have values of 6—16 (Me = 10), 4.5—12.5 (Me = 7.75), and 6—14 (Me = 9.5). *Lutzia halifaxii* has 20.5—32 (Me = 25.5) prealar setae that is significantly different from three *Culex* species listed above with $p' < 0.001$ in all comparisons as well as from *Culex hortensis*, which has 6.5—14 (Me = 9) prealar setae. *Coquillettidia richiardii* has 12.5—29.5 (Me = 22.5) prealar setae that is significantly higher than in *Culex modestus* and *Culex territans* ($p' < 1 \cdot 10^{-9}$ in both comparisons performed). *Uranotaenia unguiculata* has a single prealar seta.

Upper mesepisternal setae in majority of the specimens studied are arranged in a bow or a slanting line and located in the central region of mesokatepisternum. *Anopheles claviger* has significantly greater median number of these setae than *A. pulcherrimus* with $p' < 1 \cdot 10^{-4}$: the values are 3—10 (Me = 7.25) and 3—5 (Me = 3.75), respectively. A single specimen of *Anopheles claviger* (♂, Crimea) had a combined, continuous group of upper and lower mesepisternal setae and was excluded from the analysis. In *Coquillettidia richiardii*, 2.5—5.5 (Me = 4) upper mesepisternal setae are arranged in a horizontal line. *Culex modestus*, *C. hortensis*, *C. territans*, *C. pipiens*, and *C. theileri* have 1—2.5 (Me = 1.5), 1—2.5 (Me = 2), 3.5—6.5 (Me = 5), 3.5—7 (Me = 5.5), and 4.5—7.5 (Me = 5.5) upper mesepisternal setae, respectively. These setae are arranged in a horizontal line in case of *Culex modestus* and as a bow in *C. territans*, *C. pipiens*, and *C. theileri*. A combined group of upper and lower mesepisternal setae was found in a single male of *C. pipiens* and this case was excluded from further analysis. When *Culex modestus* was compared by median number of the upper mesepisternal setae with the latter three *Culex* species in their listing order, the differences were found to be significant with $p' < 1 \cdot 10^{-5}$ or lower. *Lutzia halifaxii* has a bow-shaped line of 6—9 (Me = 7) upper mesepisternal setae; their number differs significantly from that in *Culex modestus*, *C. hortensis*, *C. territans*, and *C. pipiens* with $p' < 0.001$ or lower. *Culiseta a. alaskaensis*, *C. a. indica*, and *Uranotaenia unguiculata* have a bow-shaped line of the upper mesepisternal setae with their ranges: 5.5—10 (Me = 6.5), 2.5—6.5 (Me = 5.25), and 4.5—7 (Me = 6), respectively.

Lower mesepisternal setae in the majority of specimens studied are arranged in 1—3 lines along the posterior edge of the mesokatepisternum. *Anopheles claviger* has significantly greater median number of setae compared to *A. pulcherrimus* ($p' < 1 \cdot 10^{-5}$): 6—13 (Me = 8.25) against 3—7 (Me = 4.25), respectively. In *Coquillettidia richiardii*, the number of lower mesepisternal setae is 9.5—18.5 (Me = 13), that is significantly higher than that in *Culex modestus* and *Culex territans* with $p' < 1 \cdot 10^{-9}$ in both comparisons; the values for these two *Culex* species are 2—13 (Me = 5.5) and 3.5—11 (Me = 7), respectively. *Culex territans* differs significantly by this parameter from *Culex theileri* ($p' < 0.001$), which has 6—14 (Me = 11.5) setae. *Culiseta alaskaensis* has 5 to 21.5 lower mesepisternal setae (differs significantly from *Anopheles claviger* with $p' < 1 \cdot 10^{-5}$). A single specimen of *Culiseta a. alaskaensis* (♀, Caucasus) had the lower mesepisternal setae combined in a common group with the upper mesepisternal setae (a total number of setae was 22—24), and was excluded from the analysis. *Lutzia halifaxii* and *U. unguiculata* have 12.5—18.5 (Me = 15.5) and 4—9.5 (Me = 6.5) lower mesepisternal setae, respectively.

Upper mesepimeral setae are arranged in an irregular group in most cases or in a bow-shaped line (in male *C. richiardii*, e. g.). *Anopheles claviger* again has

significantly greater median number of setae than *A. pulcherrimus* ($p' < 1 \cdot 10^{-6}$): the values are 12—28 (Me = 19.5) and 4—13.5 (Me = 8.5), respectively. *Culex modestus* differs significantly from *C. hortensis* by the number of upper mesepimeral setae (both species are sexually dimorphic by this parameter, see the values below) with $p' < 1 \cdot 10^{-4}$, and from *Lutzia halifaxii* with $p' < 0.001$. *Culex pipiens* differs significantly from *Lutzia halifaxii* with $p' < 0.001$; the values for these two species were 4—17 (Me = 9) and 15.5—24.5 (Me = 19), respectively. *Coquillettidia richiardii* and *Culiseta alaskaensis indica* were found to be sexually dimorphic by this parameter (see the values below). The values for *Culiseta alaskaensis alaskaensis* and *Uranotaenia unguiculata* were 15.5—29.5 (Me = 22.5) and 5—9 (Me = 7), respectively.

Lower mesepimeral setae are typically arranged in a line along the anterior border of the mesanepimeron, that is a trait of *Coquillettidia richiardii* (females), *Culiseta alaskaensis*, and *Lutzia halifaxii*. All five species of the genus *Culex* typically have a single lower mesepimeral seta, which is often located distantly from the lower border of the mesanepimeron. A single *Culex pipiens* female had a second mesepimeral seta located upward from the first seta, and two specimens (male and female) had such additional seta only on the left sclerite. Some specimens of *Culex territans* and *Culex hortensis* also possessed additional lower mesepimeral setae. A single lower mesepimeral seta is also typical of *Uranotaenia unguiculata*. In both species of the genus *Anopheles*, the lower mesepimeral setae are absent.

Sexually dimorphic setation

Under this subheading, the results of min—max ranges (and medians) calculated using averages for the left and right sides of each specimen are presented.

In *Coquillettidia richiardii*, the numbers of the upper mesepimeral setae significantly differ in males and females: ♂ 5.5—11 (Me = 7.25), ♀ 11—25.5 (Me = 19) with $p' < 0.05$. The lower mesepimeral setae are absent in the majority of males: in 2 males (from Kherson region, Ukraine), the right sclerite was equipped with 2 and 3 setae, while its right counterpart with 1 and 0 setae, respectively. Females have 2—5 (Me = 3) lower mesepimeral setae.

The median number of the lower mesepisternal setae is smaller in *Culex modestus* males than in females: 2—6.5 (Me = 5.5) and 4—13 (Me = 6.5), respectively ($p' < 0.05$). Also, males significantly differ from females by the number of the upper mesepimeral setae: 4.5—7 (Me = 5.25) and 6.5—11.5 (Me = 8.5), respectively ($p' < 0.05$).

The significant sexual dimorphism was found in the number of the postpronotal setae: 7—8.5 (Me = 7.5) and 7.5—10.5 (Me = 9.75) in *C. theileri* males and females, respectively ($p' < 0.05$).

The number of upper mesepimeral setae in *C. hortensis* significantly differs: ♂ 7—13 (Me = 10.5), ♀ 13—27 (Me = 22.5) ($p' < 0.05$).

Both sexes of *Culiseta alaskaensis indica* differ significantly from each other by the number of the prealar setae: ♂ 9—13.5 (Me = 11.25), ♀ 16—23 (Me = 19.5) ($p' < 0.05$). Furthermore, in this subspecies, the number of the upper mesepimeral setae significantly differs: ♂ 9—15.5 (Me = 11.75), ♀ 16.5—21 (Me = 18.5) ($p' < 0.05$).

Sexual dimorphism of *Culiseta alaskaensis alaskaensis* was confirmed statistically only for the lower mesepimeral setae: ♂ 2—4.5 (Me = 2.5), ♀ 4—7.5 (Me = 5.5) ($p' < 0.05$).

Asymmetry

The distribution of setae in certain setae locations were found to be very considerably asymmetric in many species studied, with statistically significant leftward biases in two species. In *Coquillettidia richiardii*, 31 of all 39 bilaterally available specimens have lateralized distribution of postpronotal setae with laterality indices ranging from -0.143 (rightward asymmetry) to $+0.231$ (leftward asymmetry) with significant leftward bias in this species ($p' = 0.026$, Wilcoxon signed rank test with continuity correction, fig. 3, A). Eight specimens appeared to be symmetric for this parameter. In *Culex modestus*, 15 of all 19 bilaterally available specimens have lateralized distribution of lower mesepisternal setae with laterality indices ranging from -0.143 (rightward asymmetry) to $+0.5$ (leftward asymmetry, the case of 3 setae on the left sclerite and 1 seta on the right sclerite). The species is significantly lateralized leftward according to this parameter ($p' = 0.046$, Wilcoxon signed rank test with continuity correction, fig. 3, B). Four specimens appeared to be symmetric for this parameter.

With $p = 0.05$ (not corrected with Holm-Bonferroni method), *Culiseta alaskaensis alaskaensis* was found to have a leftward lateralization of postpronotal setae distribution (laterality indices ranged from -0.120 to $+0.143$; 8 of 19 specimens were symmetric, fig. 3, C) — this «significant» bias may be real or just a consequence of repeated (multiple) comparisons performed on the same sample (Glantz, 1999). Further analysis of a larger number of specimens is needed.

DISCUSSION

Thoracic pleurite chaetotaxy traits play an important role in the discrimination of the Culicidae genera and species. There are stable traits characterizing certain genera. For example, the postpronotal setae are absent in *Anopheles* species, that allows to differentiate them from *Chagasia* species (Mattingly, 1973). The presence of prespiracular setae with the absence of postspiracular setae is typical of the genera *Culiseta* and *Topomyia* Leicester, 1908; the absence of prealar setae is typical of *Sabethes* species, and the presence of postspiracular and lower mesepimeral setae is typical of *Armigeres* Theobald, 1902. The subgenus *Stethomyia* Theobald, 1902, of the genus *Anopheles* is characterized by the absence of prespiracular and prealar setae (Edwards, 1932). The number of the prealar setae and the mesepisternal setae is used for species identification of the genus *Uranotaenia* (Peyton, 1977). Some subgenera and species of the genus *Culiseta* are characterized by differences in the number of prespiracular setae (Edwards, 1932; Maslov, 1967). To discriminate *Culex* species, the number of the lower mesepimeral setae was used (Gutsevich et al., 1970; Tanaka et al., 1979).

In our study, we found setation traits that were known earlier for the genus *Anopheles*: the absence of the postpronotal (Edwards, 1932) and lower mesepimeral setae (Belkin, 1962a, b). The difference between *Anopheles claviger* and

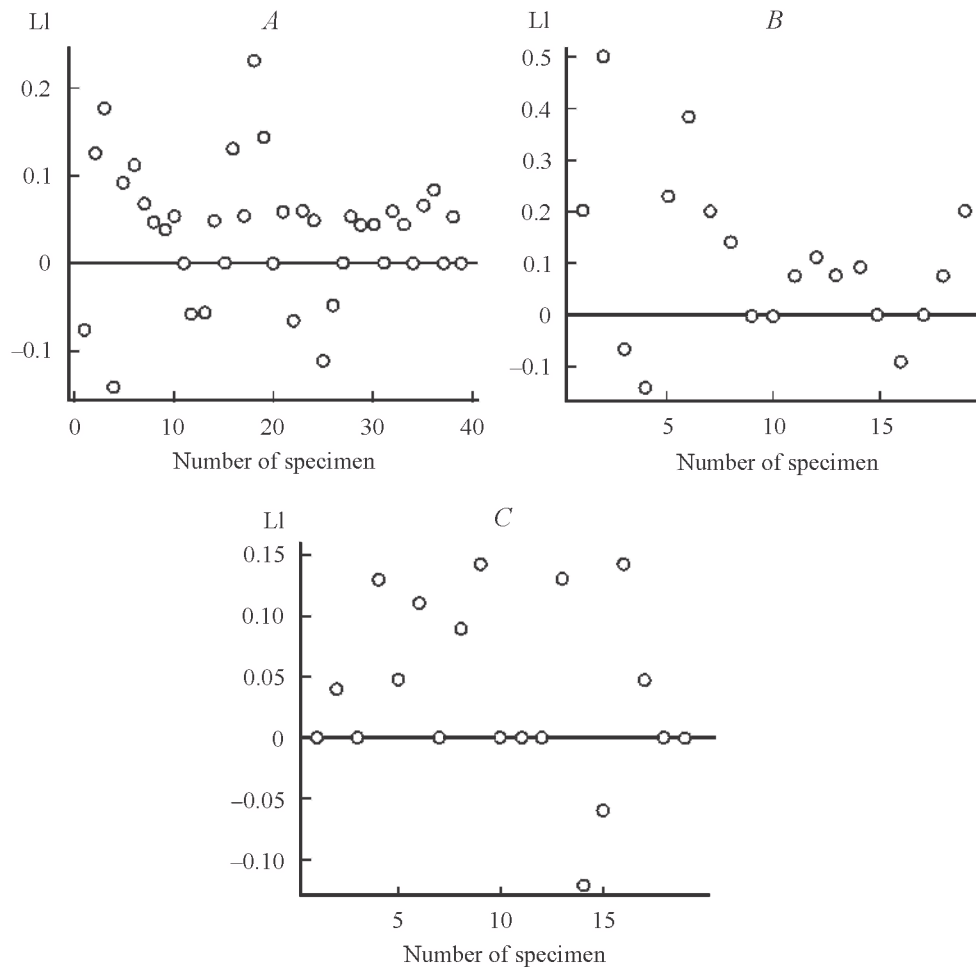


Fig. 3. Laterality indices [LI, (left-right)/(left+right)] calculated for postpronotal setae in *Coquillettia richiardii* (A), lower mesepisternal setae in *Culex modestus* (B), and postpronotal setae in *Culiseta a. alaskaensis* (C).

In the first two cases, the leftward lateralization is significant (p' less than 0.05 by Wilcoxon signed rank test with continuity correction; p-value is corrected by Holm-Bonferroni method).

A. pulcherrimus by presence/absence of prespiracular setae is a new feature currently found for *Anopheles* genus. In addition, we found that these two species significantly differ by the number of the upper and lower mesepisternal setae and by the number of the upper mesepimeral setae.

According to literature data, the genus *Uranotaenia* has 1 or more postpronotal setae, 1 to 3 (rarely more than 3) prealar setae, 0 to 2 prespiracular, and 1 to 2 lower mesepimeral setae. Our finding was that in *U. unguiculata*, a single seta represents each of the postpronotal, prealar, prespiracular, and the lower mesepimeral setae groups (with a single exception for prespiracular setae, mentioned above). In all other Culicidae that we examined, the number of setae in the first three setae groups listed above was more than 1 and/or not equal to 1.

Species of the genus *Culiseta* differ from many other species of the family Culicidae by the presence of prespiracular setae (Gutsevich et al., 1970; Becker et al., 2010). As mentioned above, we found the prespiracular setae also in *Anopheles* and *Uranotaenia* genera. Also, our finding was that *Culiseta alaskaensis* significantly differs from *Anopheles claviger*, *A. pulcherrimus*, *Culex modestus*, *C. pipiens*, *C. territans*, and *Uranotaenia unguiculata* by the greater numbers of prealar, upper and lower mesepisternal, and lower mesepimeral setae. Differences between subspecies of *Culiseta alaskaensis* were also found in our study (fig. 4, see ins.).

Species of the genus *Culex* that we studied are usually characterized by a single lower mesepimeral seta, but this feature is not typical of the whole genus *Culex* (Belkin, 1962a). Although *Uranotaenia unguiculata* also has a single lower mesepimeral seta, the *Culex* species differ from this species by greater numbers of postpronotal and prealar setae.

For the first time we found significant differences between sexes in the number of the upper mesepimeral setae in *Coquillettidia richiardii*, *Culex modestus*, *Culex hortensis* (fig. 5, see ins.), and *Culiseta alaskaensis indica*. As well, we found the significant difference between *Culex theileri* sexes in the case of postpronotal setae (fig. 6, see ins.).

Most of the studied species significantly differ from each other by setation. Nevertheless, there is a notable overlap between the species, when certain group of setae is considered. In many such cases, the number of setae in a certain group of setae cannot serve as a single reliable criterion to discriminate the species. But these characters may be used to develop the probabilistic approach to discriminate different species with various overlaps of setae numbers in several setae groups. Such approach could be needed, in particular, when damaged specimens are studied.

In the present study we found that in many cases the number of setae in certain groups on the left and right sides of the thorax are unequal, therefore we emphasize our interest in the question: may asymmetric setation noted in most of specimens be critically important and taken into account in the process of species diagnostics?

In most specimens, the differences between the left and the right parts are not so great, staying within the range of 1 to 3 setae. Nevertheless, differences of such range lead to quite high laterality index, if the number of setae per group is not high. We found statistically confirmed asymmetry for certain groups of setae on the species level for *Coquillettidia richiardii* and *Culex modestus*.

In general, we found that, when the number of specimens in a sample was not very small, it was difficult to find a mosquito species in which even a single group of setae was lateralized in all cases. For instance, in cases where the sample size was more than 10 individuals, only in *Culex territans* specimens ($n = 11$) with both intact sites of the thorax, the asymmetrically distributed upper mesepisternal setae were present in all cases. Also it is difficult to find a mosquito species with the laterality of only one of two possible directions. Again, only in case of *Culex territans*, we found that the additional, second lower mesepimeral seta is located on the left side in all 4 asymmetric specimens in a total sample of 13 specimens. Using *Culex territans* as an example, if we used a quite small sample size ($n = 11$ —13), the probability of finding the symmetric distribution of the upper mesepisternal setae remains substantial, and the probability

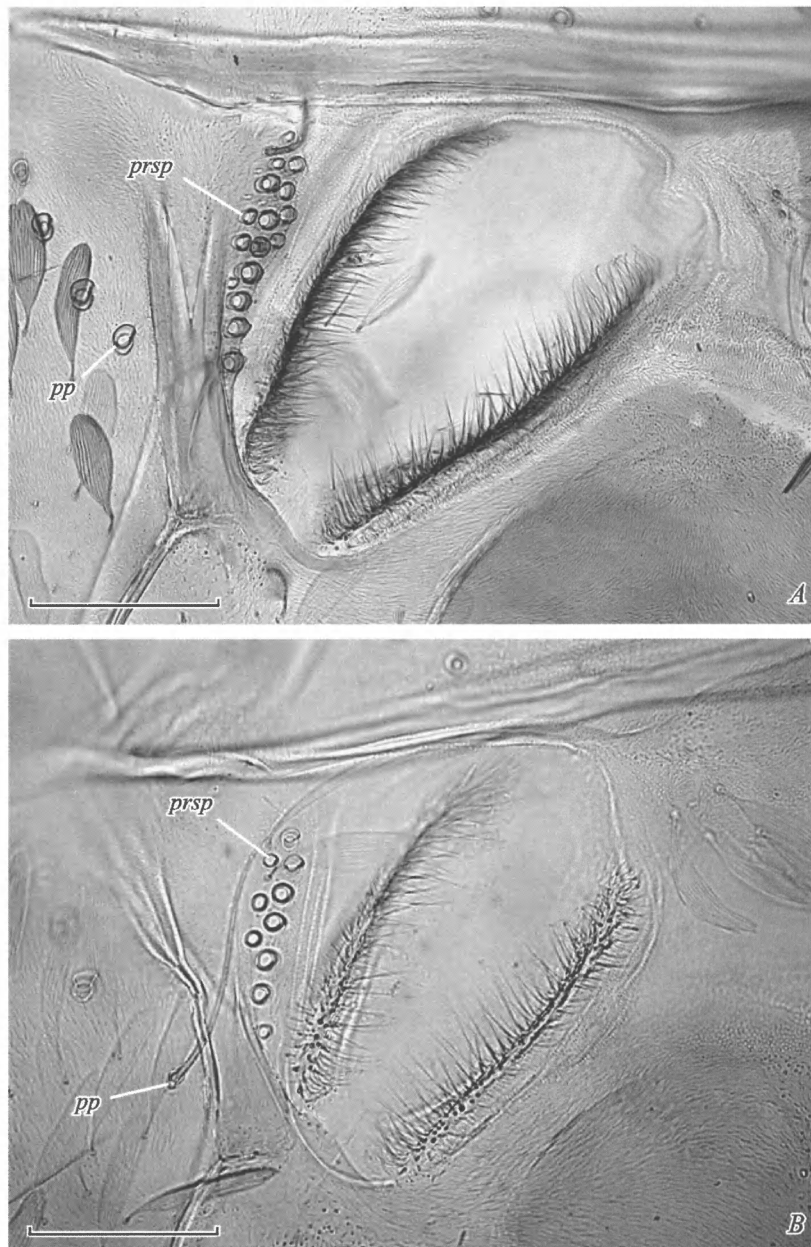


Fig. 4. *Culiseta alaskaensis* (Ludlow, 1906), 1st thoracic spiracle area, lateral view by LM.
 A — *C. a. alaskaensis*, B — *C. a. indica* (Edwards, 1920). Scale bars — 0.1 mm. Designations are as in fig. 2.

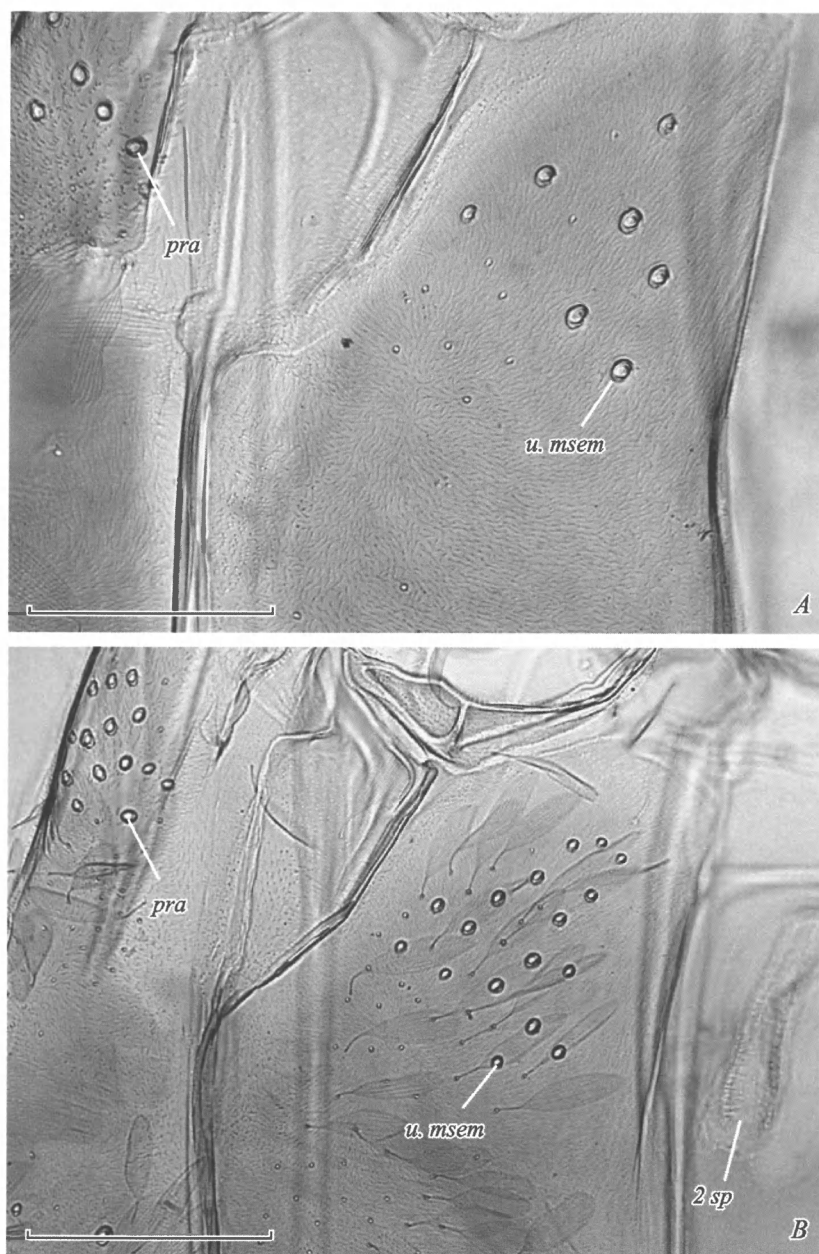


Fig. 5. *Culex hortensis* Ficalbi, 1889, mesokatepimeron, lateral view by LM.
A — ♂, B — ♀. Scale bars — 0.2 mm. Designations are as in fig. 1, 2.



Fig. 6. *Culex theileri* Theobald, 1903, postpronotum, lateral view by LM.
 A — ♂, B — ♀. Scale bars — 0.1 mm. Designations are as in fig. 1, 2.

to find the right-lateralized distribution of the lower mesepimeral setae also remains substantial. In other words, the unobserved cases cannot be declared as rare, not typical, or (moreover!) not existing in this species, because the amount of the specimens studied was small.

Considering the obtained results, we can suggest that the asymmetry of setation is not a factor critically affecting the species diagnosis by setation. Nevertheless, the setation asymmetry may be very useful in elaboration of the methods using the determination of probability of species belonging if the similar species (overlapping by setation) are analyzed and/or the damaged material with the setation of only one available side is studied.

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References

- Becker N., Petric D., Zgomba M., Boase C., Madon M., Dahl C., Kaiser A. 2010. Mosquitoes and their control. Second Edition. Berlin Heidelberg: Springer-Verlag. 608 p.
- Belkin J. N. 1962a. The mosquitoes of the South Pacific (Diptera, Culicidae). University of California Press, Berkeley and Los Angeles. 1. 608 p.
- Belkin J. N. 1962b. The mosquitoes of the *South Pacific* (Diptera, Culicidae). University of California Press, Berkeley and Los Angeles. 2. 412 p.
- Edwards F. W. 1932. Diptera, fam. Culicidae. Brussels: Desmet-Verteneuil, 258 p. (Genera Insectorum, Fascile. 194).
- Glantz S. 1999. Mediko-biologicheskaya statistika [Primer of biostatistics]. Moscow: Praktika. 459 p. (In Russian).
- Gutsevich A. V., Monchadsky A. S., Schtakelberg A. A. 1970. Komary, semeystvo Culicidae [The mosquitoes, family Culicidae]. Fauna SSSR, nasekomye dvukrylye [Fauna of the USSR, Insects, Dipterans]. L.: Nauka. 3 (4): 384 p.
- Holm S. A. 1979. A Simple Sequentially Rejective Multiple Test Procedure. Scandinavian Journal of Statistics. 6 (1): 65—70.
- Huber B. A., Sinclair B. J., Schmitt M. 2007. The evolution of asymmetric genitalia in spiders and insects. Biological Reviews. 82 (4): 647—698.
- Khalin A. V., Aibulatov S. V. 2012. New investigation technique for thoracic sclerites of mosquitoes (Diptera, Culicidae) for the correct identification of genera and species. Entomological review. 92 (9): 988—993.
- Khalin A. V., Aibulatov S. V. 2014. English and Russian terminology for the thoracic skeletal structures of mosquitoes (Diptera: Culicidae): a critical review. Entomological Review. 94 (7): 959—974.
- Khalin A. V., Aibulatov S. V. 2016. Diagnostic characters of the thoracic pleurites of mosquitoes (Diptera, Culicidae): Topology of setae in species of the genera *Anopheles* Meigen, 1818, *Coquillettidia* Dyar, 1905, *Culex* Linnaeus, 1758, *Culiseta* Felt, 1904, *Lutzia* Theobald, 1903, and *Uranotaenia* Lynch Arribalzaga, 1891. Entomological Review. 96 (8): 1041—1068.
- Leggewie M., Badusche M., Rudolf M., Jansen S., Börstler J., Krumkamp R., Huber K., Krüger A., Schmidt-Chanasit J., Tannich E., Becker S. C.

2016. *Culex pipiens* and *Culex torrentium* populations from Central Europe are susceptible to West Nile virus infection. *One Health*. (2): 88—94.
- L'vov D. K., Klimenko S. M., Gaydamovich S. Ya. 1989. *Arbovirusy i arbovirusnyie infektsii* [Arboviruses and arbovirus infections]. Moscow: Medicine. 334 p. (In Russian).
- Maslov A. V. 1967. Krovososushchie komary podtriby Culisetinae (Diptera, Culicidae) mirovoj fauny. Opredeliteli po faune SSSR [Blood-sucking mosquitoes of the subtribe Culisetinae (Diptera, Culicidae) in world fauna (Keys to the fauna of the USSR)]. L.: Publish of USSR Academy of Sciences. 182 p. (In Russian).
- Mattingly P. F. 1973. Culicidae (Mosquitoes) [Illustrated keys to the genera of mosquitoes]. *Insects and other Arthropods of Medical Importance*. London. 37—107.
- Peyton E. L. 1977. Medical entomology studies X. A revision of the subgenus *Pseudoficalbia* of the genus *Uranotaenia* in Southeast Asia (Diptera: Culicidae). *Contributions of the American Entomological Institute*. 14 (3): 271.
- R Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: <https://www.R-project.org/>.
- Razali N. M., Wah Y. B. 2011. Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. *Journal of Statistical Modeling and Analytics*. 2 (1): 21—33.
- Razygraev A. V. 2013. Anatomical asymmetry of the retrocerebral complex in the cricket, *Gryllus locorojo* (Orthoptera: Gryllidae). *Boletín del Museo de Entomología de la Universidad del Valle*. 14 (2): 4—11.
- Tanaka K., Mizusawa K., Saugsteg E. 1979. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu archipelago and the Ogasawara islands) and Korea (Diptera: Culicidae). *Contributions of the American Entomological Institute*. 16. 987 p.